

THE SOLUBILITY IN AQUEOUS UREA SOLUTIONS OF THE MICELLAR  
CASEINATES OF MILK AND MILK PRODUCTS SUBJECTED  
TO VARIOUS STERILIZING HEAT TREATMENTS

SUMMARY

A study was made of the changes that occur in the micellar caseinates when heated in their natural environment.

The caseinates were separated from heated milks by centrifugation at 27,000 times gravity in a Servall centrifuge, followed by washing and lyophilizing. The dry caseinates were equilibrated by shaking for 24 hr. at 25° C. in 6.6 *M* urea solution. After dilution to 3.3 *M* urea, and equilibration for 48 hr., the solutions were centrifuged to separate the insoluble caseinates. The soluble caseinates of the solution were determined by micro-Kjeldahl analyses after dialysis to remove urea. Solubility curves, obtained by plotting the amount of the casein in 3.3 *M* urea solution against the amount of micellar caseinates present in the system, exhibit solubility phenomena characteristic of heterogeneous systems and show that:

1. Increased heating decreases the solubility of the caseinates in aqueous urea.
2. Fat present in milk during heating decreases the solubility in urea.
3. Increased solids content of milk gives decreased protein solubility in aqueous urea after heating.
4. High-temperature—short-time methods of sterilizing concentrated milk products generally produce less change in the protein solubility in urea than does the conventional sterilization method.
5. The presence of citrate and phosphate ions in the urea system increases the protein solubility.
6. The heat-produced protein component in the casein micelle concentrates in the 3.3 *M* urea insoluble fraction.

Heat sterilization produces a number of undesirable organoleptic and physical changes in milk products (10). The mechanism of the chemical reactions underlying these gross changes is not well understood, but many of the observed changes are thought to be associated with heat-induced modification of the milk proteins. Study of the milk proteins and the changes they undergo during heating is, therefore, of importance in the development of superior dairy products.

This paper reports results obtained during a study of the physical and chemical changes that occur in the casein fraction of milk and milk products during sterilizing heat treatments.

Casein (8), which is the principal protein fraction of milk (2), has been shown to exist in the forms of large micelles of complex composition (7). Various methods have been devised to isolate and fractionate these micellar proteins (4, 5, 11, 12). Up to the present at least five different caseins have been found in the casein micelle, in addition to inorganic ions and colloidal salts.

Experimental work using alpha and beta caseins, two of the more important constituents of the micelle, has been interpreted to indicate that these caseins

have no specific three-dimensional space configuration and can, therefore, be considered to act as denatured globular proteins (3). If this is true, then the caseins should not be denatured by high heat treatment in neutral solutions. However, other work (6, 13, 14) has shown that heat does change the physical and chemical properties of the caseins isolated from milk. The changes were reported to be both reversible and irreversible, depending on the severity of the heat treatment employed. Since this earlier work was carried out in simplified model systems, it was considered desirable to study the changes that occur in the micellar proteins of milk when heated in their native environment, wherein the possibility of reaction with the other constituents of milk exists. Previously reported work (9) had shown that an electrophoretically unique protein (*X*), which had a mobility of  $6.0 \times 10^{-5}$  cm.<sup>2</sup> volt<sup>-1</sup> sec.<sup>-1</sup>, appears in the casein micelle of heat-sterilized milks. To facilitate the eventual isolation of this protein, a solubility study of the micellar proteins isolated from such heated milks by centrifugation was made. Solutions of 3.3 *M* urea were used to determine maximum differences in solubilities of the caseinates. This concentration of urea produces a median solubility between insolubility of these caseinates in water and complete solubility in 6.6 *M* urea solutions.

#### EXPERIMENTAL PROCEDURE

*Preparation of material.* The micellar casein complex used for the determination of solubility was sedimented from the appropriate milk product by centrifuging for 1 hr. in a Servall super-speed angle-head centrifuge at a speed of about 14,000 r.p.m. and a centrifugal force of approximately 27,000 times gravity. The caseinate complex was washed by redispersing in distilled water, using a mortar and pestle or Waring Blendor. After two washings the redispersed caseinate was hard-frozen in metal trays, and the material was lyophilized in a pilot plant model of a Stokes shelf dryer. The dried complex was ground to a fine powder using a mortar and pestle. It should be noted that even at the low centrifugal force used in this preparation there is the possibility of the inclusion of small amounts of heat-aggregated whey proteins with the sedimented caseinates.

*Determination of solubilities.* In each series of solubility experiments, sufficient of the dried caseinate complex was placed in a 50-ml. plastic centrifuge tube and enough water added to make 15 ml. of solution containing 2, 4, 6, 8, 10, 12, 14, and 18 g. per 100 ml. Sufficient urea had previously been added to make the solution 6.6 *M* when made up to the 15-ml. mark. The tubes were shaken for 24 hr. at 25° C., diluted with water to the 30-ml. mark to bring the urea concentration to 3.3 *M*, and shaken for an additional 48 hr. to insure equilibration. The pH of the solutions was  $7.3 \pm 0.2$ . The tubes were then centrifuged in the Servall centrifuge for 1 hr. at 14,000 r.p.m. Aliquots of the clear solutions containing the dissolved caseinates were placed in small dialysis sacks and the contents dialyzed against distilled water until no urea was shown to be present. The aqueous contents were pervaporated, the dried

sack and contents digested, and nitrogen and protein determined by the conventional Kjeldahl micro-method.

The distribution of the several components of the original caseinate complex in that portion dissolved by the 3.3 *M* urea, and in the portion undissolved by urea, was determined by taking aliquots of the above clear liquid and of the sedimented and undissolved portion and dialyzing to equilibrium against veronal buffer containing 0.05 *M* NaCl at pH of 8.4 and ionic strength of 0.1. After clearing the dialyzed solution by centrifugation, an electrophoretic analysis was conducted in a portable Aminco apparatus, using a standard 8-ml. analytical cell. The photographic patterns were projected and traced on graph paper. Protein distribution and mobilities were measured, using conventional techniques (1).

The effect of 0.1 *M* citrate and of 0.1 *M* phosphate on the solubility of the micellar caseinates centrifuged out of heated skimmilk was studied in a similar manner.

#### RESULTS

The solubility curves obtained by plotting the amount of soluble casein against the amount of micellar caseinate present in the system have forms characteristic of heterogeneous systems and are shown in Figures 1-4. A comparison of these curves shows the effects of various processing and chemical factors on the solubilities of the caseinate micelles.

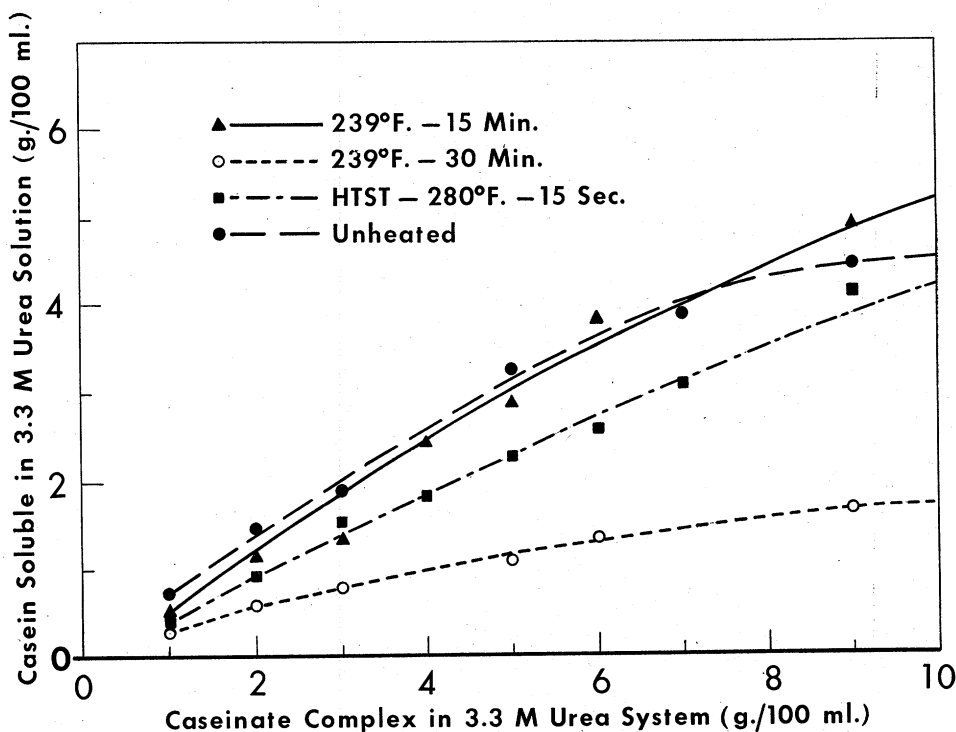


FIG. 1. Effect of heat treatment of milk on caseinate solubilities.

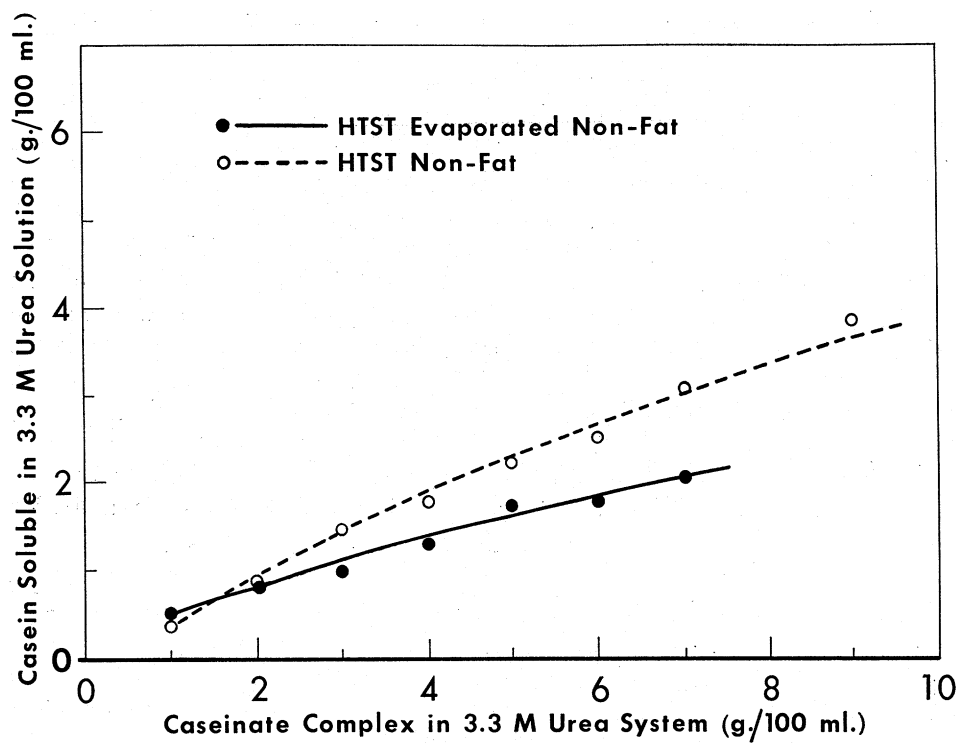


FIG. 2. Effect of solids concentration on caseinate solubilities.

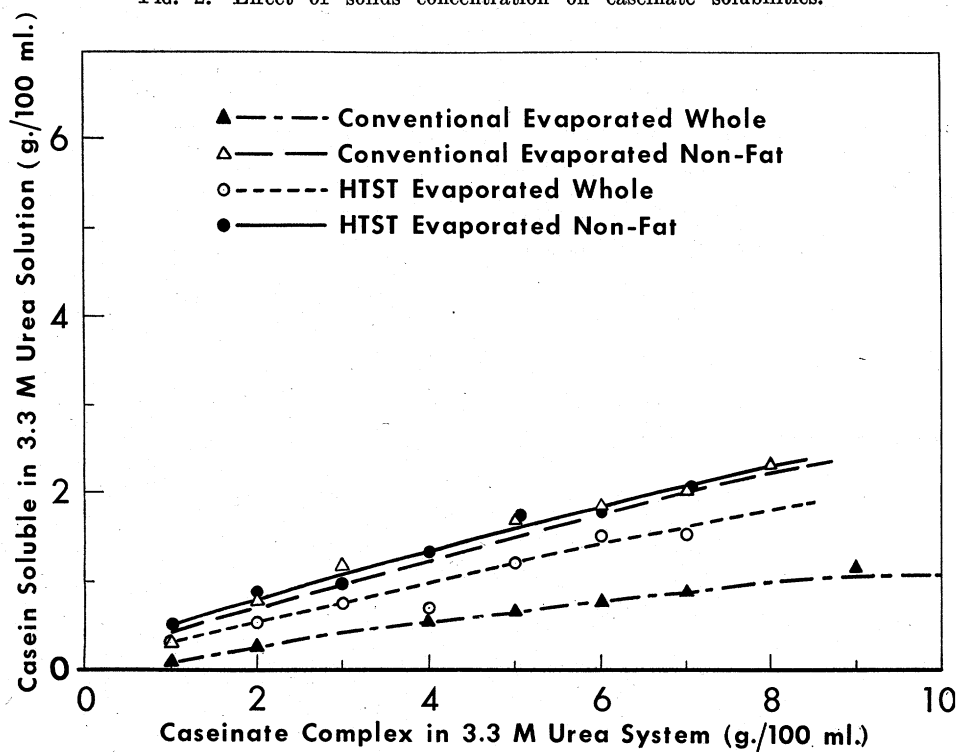


FIG. 3. Effect of fat content and of sterilizing methods on caseinate solubilities.

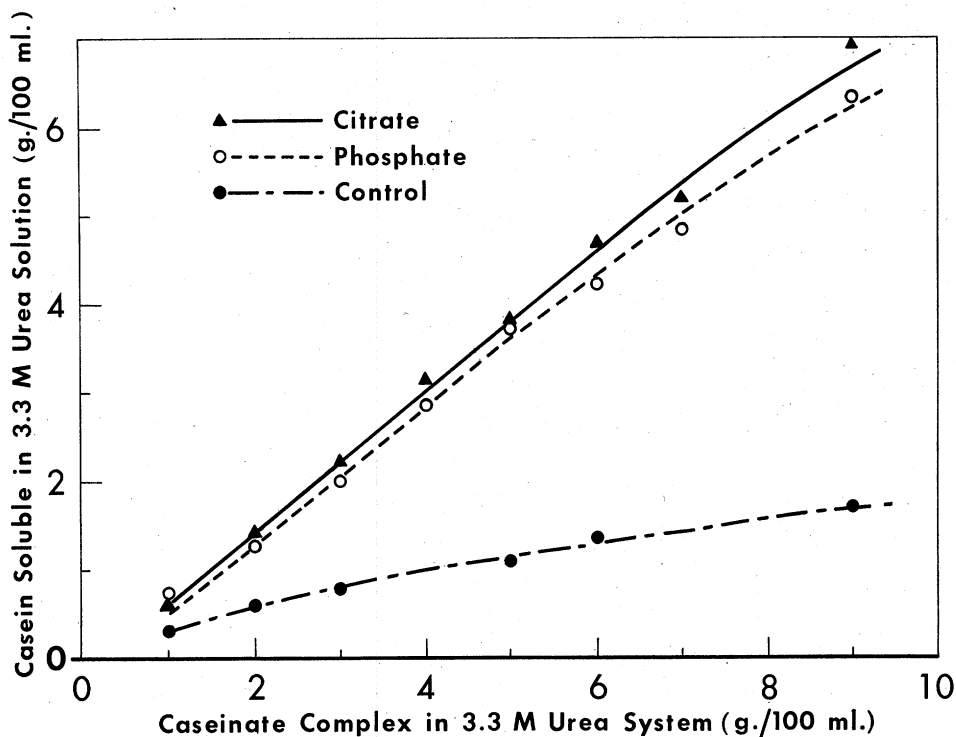


FIG. 4. Effect of phosphates and citrates on caseinate solubilities. Milk at 239° C.—30 min.

The effect of intensity and amount of heat treatment on the solubilities of caseinates from normal strength skim milk is shown in Figure 1. High heat treatment produces caseinates of low solubility, H.T.S.T. (280° F. for 15 sec.) treatment produces those of intermediate solubility, and the caseinates subjected to low heat treatment are practically as soluble as those from unheated skim-milk. The effect of solids concentration on the solubility of caseinates in urea solutions is shown in Figure 2. High-temperature—short time heat treatment on single-strength nonfat milk produces caseinates of higher solubility than does the same heat treatment on double-strength evaporated nonfat milk.

A comparison of the two conventional evaporated milk curves in Figure 3 shows that the presence of fat during the conventional process of heating of 2:1 evaporated milk decreases the solubility of the caseinates. Similarly, the H.T.S.T. curves on this same figure show the same results. By comparing the two H.T.S.T. evaporated curves with the two conventional evaporated curves, the effect of the method of sterilization of evaporated milk on the solubility of the caseinates is shown. It is evident that the H.T.S.T. method of sterilization has less effect on the solubility of the caseinate in urea than does the conventional method, especially in the case of the evaporated whole milk. The difference in the effect of the two methods of sterilization is less apparent for the evaporated nonfat milks.

The increase in solubility in urea solution of the caseinates from severely heated milk in the presence of citrate and of phosphate ions is shown in Figure 4.

TABLE 1  
Electrophoretic distribution of proteins in 3.3 molar urea systems

Type of milk and heating	Phase							
	Soluble				Insoluble			
	$\alpha$ Casein	X	$\beta$ Casein	$\gamma$ Casein	$\alpha$ Casein	X	$\beta$ Casein	$\gamma$ Casein
	(%)							
H.T.S.T. evaporated whole	53.4	8.9	28.3	8.9	95.0	5.0	0.0	0.0
H.T.S.T. evaporated skim	45.8	14.7	30.1	9.4	55.3	44.7	0.0	0.0
Conventional evaporated whole <sup>a</sup>	39.5	35.3	25.2	0.0	100.0	0.0	0.0	0.0
H.T.S.T. Skimmilk	72.5	5.5	22.0	0.0	83.5	10.1	6.4	0.0
Skim—115° C. 30 min.	59.0	11.0	17.3	12.7	69.0	22.0	8.9	0.0
Skim—115° C. 15 min.	66.6	11.6	18.8	3.0	75.0	8.6	16.4	0.0

<sup>a</sup> Very little of the insoluble phase was capable of being solubilized by veronal buffer in preparation for electrophoretic analysis and, therefore, the percentages given probably do not represent a true distribution of the various components.

Table 1 shows some typical results on the electrophoretic distribution of the various casein components between the 3.3 *M* urea soluble and insoluble fractions. In connection with the possibility of the inclusion of heat-coagulated proteins mentioned previously, it should be noted that the various casein fractions may contain some of these whey proteins, even though the mobilities actually found are normal for the casein fractions indicated. The beta casein and gamma casein components have a disposition to concentrate in the urea-soluble fraction while alpha casein and the heat-produced *X* component have a tendency to concentrate in the urea-insoluble fraction. This tendency may make it possible for the *X* component to be separated from the other components by convection electrophoresis or other methods of purification, so that its identity and composition may be studied.

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